Citation:

Lankinen M, Schwab U, Erkkilä A, Seppänen-Laakso T, Hannila ML, Mussalo H, Lehto S, Uusitupa M, Gylling H, Oresic M. Fatty fish intake decreases lipids related to inflammation and insulin signaling--a lipidomics approach. *PLoS One.* 2009;4(4):e5258. Epub 2009 Apr 23.

PubMed ID: 19390588

Study Design:

Randomized Controlled Trial

Class:

A - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

NEUTRAL: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

The aim of this study was to investigate how fatty fish or lean fish in a diet affect serum lipidomic profiles in subjects with coronary heart disease.

Inclusion Criteria:

- Identified from the discharge lists of the Kuopio University Hospital
- Acute myocardial infarction or unstable ischemic attack during the previous 3-36 months
- Age under 70 years
- Normal sinus rhythm
- Fasting serum triglyceride concentration ≤3.5 mmol/l
- Fasting serum cholesterol concentration ≤8 mmol/l
- Body mass index (BMI) 18.5-30 kg/m^2
- Fasting plasma glucose concentration ≤7.0 mmol/l
- Had to use beta-blockers

Exclusion Criteria:

- Use of other antiarhythmic medications than beta blockers or psychotropic drugs
- Diagnosed with diabetes, atrial fibrillation, inflammatory bowel disease or abnormal liver, thyroid or kidney function
- Excessive amounts of alcohol
- Reported use of fish oil supplements or high fish intakes (>3 meals/week) during the last three months

Description of Study Protocol:

Recruitment

Subjects were identified from the discharge lists of the Kuopio University Hospital.

Design

The study was an 8-week randomized, controlled, parallel pilot study.

Blinding used (if applicable): not noted

Intervention (if applicable)

- Fatty fish group: consume fish 100-150 g/meal at least four times a week: salmon, rainbow trout, Baltic herring, whitefish and vendace
- Lean fish group: consume fish 100-150 g/meal at least four times a week: pike, pike-perch, perch, saithe and cod
- Control group: consume less than 1 fish meal per week and to eat meals made with lean meat (beef or pork) or chicken without skin.
- All subjects followed a diet recommended for CHD patients and were advised to avoid sources of saturated fat in food preparation

Statistical Analysis

- Biochemical data were statistically analyzed using the SOSS statistical software (version 14.0, SPSS Inc, Chicago, IL) and R software version 2.4.1.
- The data are expressed as mean ± SD.
- Fold changes for the lipids were calculated dividing the endpoint values by the baseline values.
- The HOMA insuling resistance index (HOMA-IR) was calculated.
- The normality of distributions of the variables was tested with Kolmogorov-Smirnov test with Lilliefors signficance correction.
- The variables with abnormal distribution were normalized with logarithmic transformation or a non-parametic test was used if normal distribution was not achieved with transformation.
- Paired samples *t*-test or Wilcoxon signed ranks test was used when comparing baseline and endpoint values within the groups.
- Spearman rank correlation coefficients, *P*-values <0.05 were considered significant.
- For the high-dimensional lipidomics dataset, the *P*-values were corrected for multiple comparison by calculating the False Discovery Rate (FDR) *q*-values.
- In order to compare the group differences at baseline and after the intervention, one-way analysis of variance (ANOVA) was performed on lipidomics data for each lipid separately at baseline and after the intervention.
- The FDR *q*-values were also calculated from the ANOVA *P*-values.
- *q*-values <0.05 were considered significant.
- Mixed model was used to assess the relation between lipidomics variables and sum of EPA and DHA.

Data Collection Summary:

Timing of Measurements

At baseline and week 8.

Dependent Variables

- Serum lipids: lipidomic analyses were performed using ultra performance liquid chromatography coupled to electrospray ionization mass spectrometry and gas chromatography.

Independent Variables

- Fatty fish group: 100-150 g/meal at least 4 times per week: salmon, rainbow trout, Baltic herring, whitefish and vendace

- Lean fish group: 100-150 g/meal at least 4 times per week: pike, pike-perch, perch, saithe and cod
- Control group: consume less than 1 fish meal per week and to eat meals made with lean meat (beef or pork) or chicken without skin.
- All subjects followed a diet recommended for CHD patients and were advised to avoid sources of saturated fat in food preparation
- Dietary compliance was monitored by using a 7-day food record kept twice during the intervention (weeks 3 and 7) and with the daily record of fish consumption

Control Variables

Description of Actual Data Sample:

Initial N: 44 subjects identified; 35 subjects accepted into the study, 12 in the Fatty Fish group, 12 in the Lean Fish group, 11 in the Control group

Attrition (final N): 33 subjects completed the study: 11 in the Fatty Fish group, 12 in the Lean Fish group, and 10 in the Control group

Age: under 70 years

Ethnicity: not described

Other relevant demographics:

Anthropometrics published in a previous study (not available in this document)

All subjects were using statins in addition to betablockers, and 88% of subjects were using acetosalisylic acid, 45% ACE inhibitor, 39% oran anticoagulant, 27% calcium antagonist and 27% nitrate

Location:Kuopio University Hospital, Finland

Summary of Results:

Key Findings

- Multiple bioactive lipid species, including ceramides, lysophosphatidylcholines and diacylglycerols, decreased significantly in the fatty fish group.
- In the lean fish group, cholesterol esters and specific long-chain triacyglycerols increased significantly (False Discovery Rate q-value < 0.05).

Clinical characteristics at baseline and after the 8-week intervention (mean±SD)

	Fatty Fish (n=11) 0 week	Fatty Fish 8 wk	Fatty Fish *P*-value	Lean Fish (n=12) 0 week	Lean Fish 8 wk	Lean Fish *P*-value	Control (n=10) 0 week	Control 8 wk	Control *P*-value
Body mass index (kg/m^2)	26.7±3.1	26.8±3.2	0.602	27.8±2.1	27.5±1.9	0.051	27.0±2.9	26.9±2.9	0.190
Serum cholesterol (mmol/l)	3.8±0.6	3.5±0.6	0.361	3.7±0.7	3.7±0.6	0.805	4.7±0.8	4.3±0.8	0.027

LDL cholesterol (mmol/l)	1.9±0.3	1.9±0.3	0.585	2.0±0.6	2.0±0.5	0.975	2.6±0.6	2.5±0.6	0.162
HDL cholesterol (mmol/l)	1.4±0.4	1.5±0.4	0.246	1.3±0.3	1.3±0.3	0.568	1.4±0.5	1.3±0.4	0.028
Serum triacylglycerols (mmol/l)	1.3±0.7	1.1±0.4	0.278	1.1±0.6	1.1±0.6	0.981	1.9±1.2	1.7±0.5	0.403
Plasma glucose (mmol/l)	6.1±0.9	6.1±0.5	1	5.6±0.4	5.5±0.4	0.234	5.7±0.3	5.6±0.3	0.185
Serum insulin (mU/l)	13.8±12.4	12.3±8.8	0.425	9.7±5.1	8.0±3.8	0.175	12.6±7.3	11.0±4.7	0.392
HOMA-IR	4.1±4.8	3.4±2.8	0.547	2.4±1.2	2.0±1.0	0.155	3.2±1.9	2.7±1.2	0.41

Main lipidomic changes across the three groups

Lipid Name	*q*-value 0 wk	*q*-value 8 wk	*P*(8wk) Fatty fish vs Control	*P*(8wk) Lean fish vs Control	*P*(8wk) Fatty fish vs Lean fish	Within-person Log2 Fold Fatty fish	*q*-value Fatty fish	Within-person Log2 Fold Lean fish	*q*-value Lean fish
Cer(d18:1/23:0)	0.32	0.0163	0.0060	0.3629	0.1438	**0.72**	**0.0316**	1.14	0.3434
Cer(d18:1/24:1)	0.15	0.0112	0.0100	0.1179	0.2048	**0.79**	**0.0418**	1.30	0.1861
ChoE(20:5)	0.15	0.0014	0.0069	0.7417	0.0022	**2.48**	**0.0145**	**1.53**	**0.0450**
DG(32:5)	0.33	0.0116	0.0126	0.2925	0.0406	**0.70**	**0.0350**	0.93	0.2367
DG(40:1)	0.41	0.0163	0.0242	0.9314	0.0278	**0.71**	**0.0431**	0.96	0.2776
DG(44:7)	0.33	0.0231	0.0464	0.7112	0.0488	**0.69**	**0.0340**	1.0	0.3416
PC(32:0)	0.53	0.0092	0.0112	0.4808	0.0183	**0.81**	**0.0431**	1.11	0.4004
PC(32:3)	0.34	0.0137	0.0165	0.9481	0.0244	**0.64**	**0.0046**	1.10	0.4318
PC(34:1)	0.18	0.0016	0.0009	0.0135	0.0937	**0.80**	**0.0340**	1.27	0.2251
PC(34:2)	0.25	0.0074	0.0060	0.1091	0.1285	**0.80**	**0.0340**	1.21	0.2387
PC(34:3)	0.52	0.0157	0.0360	0.8519	0.0094	**0.60**	**0.0346**	2.30	0.3328
PC(36:0)	0.30	0.0194	0.0323	0.3009	0.1201	**0.76**	**0.0219**	1.00	0.3821
PC(36:2)	0.18	0.0131	0.0283	0.0766	0.3837	**0.83**	**0.0285**	1.28	0.1559
PC(36:4)	0.15	0.0026	0.0007	0.0694	0.0466	**0.75**	**0.0145**	1.31	0.1179
PC(36:5)	0.27	0.0007	0.0031	0.7644	0.0005	**1.59**	**0.0219**	1.18	0.2147
PC(38:4)	0.14	0.0148	0.0201	0.1403	0.2482	**0.76**	**0.0144**	1.34	0.1387
PC(38:6)	0.33	0.0007	0.0000	0.0147	0.0272	**0.78**	**0.0219**	1.34	0.2222
PC(38:8)	0.24	0.0227	0.0289	0.8910	0.0598	**0.73**	**0.0215**	1.01	0.3821

PC(40:6)	0.20	0.0061	0.0017	0.0671	0.1738	**0.79**	**0.0092**	1.47	0.1324
PC(32:0e)	0.15	0.0299	0.0619	0.2177	0.4420	**0.85**	**0.0405**	1.11	0.2511
PC(32:1e)	0.15	0.0282	0.1302	0.0546	0.9694	**0.80**	**0.0260**	1.08	0.3434
PC(34:0e)	0.43	0.0074	0.0055	0.2738	0.0460	**0.74**	**0.0340**	0.99	0.3434
PC(34:3e)	0.30	0.0502	0.1275	0.4267	0.3502	**0.87**	**0.0470**	1.25	0.1731
PC(36:5e)	0.29	0.0168	0.0301	0.2182	0.1671	**0.81**	**0.0431**	1.19	0.2519
PC(36:5e)	0.29	0.0215	0.0333	0.2221	0.2432	**0.82**	**0.0431**	1.15	0.3416
PC(38:5e)	0.28	0.0073	0.0075	0.0464	0.2917	**0.80**	**0.0418**	1.19	0.3328
PC(38:6e)	0.27	0.0292	0.0437	0.6380	0.1409	**0.75**	**0.0418**	1.05	0.4004
PC(40:4e)	0.15	0.0519	0.0867	0.2897	0.6964	**0.80**	**0.0340**	1.05	0.4137
PE(32:1)	0.33	0.0225	0.0409	0.5987	0.0428	**0.73**	**0.0336**	1.11	0.4004
PE(34:3e)	0.45	0.0049	0.0027	0.1397	0.0424	**0.80**	**0.0442**	1.15	0.3699
PE(36:2e)	0.48	0.0030	0.0021	0.0576	0.0407	**0.69**	**0.0260**	1.07	0.4004
PE(36:5e)	0.52	0.0040	0.0051	0.1544	0.0144	**0.85**	**0.0418**	1.06	0.3254
PE(36:6e)	0.33	0.0028	0.0358	0.2720	0.0006	**1.29**	**0.0340**	1.10	0.4004
PE(38:5e)	0.33	0.0013	0.0004	0.0482	0.0088	**0.80**	**0.0486**	1.25	0.1221
PS(32:0)	0.42	0.0148	0.0254	0.6437	0.0202	**0.74**	**0.0486**	1.21	0.4191
lysoPC(16:0)	0.31	0.0167	0.0602	0.0413	0.8165	**0.86**	**0.0219**	1.07	0.4321
lysoPC(16:1)	0.35	0.0266	0.0665	0.0808	0.6391	**0.80**	**0.0219**	1.03	0.3821
lysoPC(18:0)	0.28	0.0416	0.2450	0.1176	0.6576	**0.84**	**0.0291**	1.11	0.4237
lysoPC(18:1)	0.34	0.0148	0.0318	0.0458	0.6183	**0.78**	**0.0260**	1.04	0.4004
lysoPC(18:1e)	0.47	0.0176	0.0435	0.0728	0.5809	**0.75**	**0.0046**	0.87	0.1308
lysoPC(18:2)	0.48	0.0585	0.1557	0.5233	0.3675	**0.80**	**0.0316**	1.01	0.3821
lysoPC(18:2e)	0.32	0.0384	0.1048	0.2481	0.5073	**0.79**	**0.0260**	1.09	0.4364
lysoPC(20:3)	0.25	0.0028	0.0005	0.1476	0.0331	**0.69**	**0.0219**	1.11	0.3434
lysoPC(20:4)	0.33	0.0316	0.0502	0.3135	0.3082	**0.73**	**0.0219**	1.03	0.4004
lysoPE(19:2e)	0.40	0.0157	0.0366	0.0576	0.6570	**0.85**	**0.0219**	1.03	0.4042
lysoPE(20:0e)	0.28	0.0058	0.0038	0.1484	0.0429	**0.60**	**0.0101**	0.90	0.2045
TG(51:2)	0.41	0.0148	0.0021	0.1537	0.3702	**0.72**	**0.0409**	1.29	0.4067
TG(52:2)	0.15	0.0007	0.0004	0.0021	0.3932	**0.77**	**0.0489**	2.08	0.1279
TG(53:2)	0.15	0.0008	0.0002	0.0028	0.3282	**0.74**	**0.0431**	1.57	0.2511
TG(54:1)	0.45	0.0083	0.0086	0.1742	0.0464	**0.72**	**0.0431**	1.14	0.3989
TG(54:2)	0.23	0.0007	0.0004	0.0013	0.4665	**0.73**	**0.0431**	1.74	0.1861
TG(55:3)	0.29	0.0023	0.0003	0.0139	0.2371	**0.75**	**0.0486**	1.35	0.3821
TG(56:4)	0.15	0.0042	0.0013	0.0739	0.1085	**0.72**	**0.0418**	1.71	0.1869
TG(56:5)	0.15	0.0007	0.0002	0.0004	0.5019	**0.74**	**0.0405**	1.88	0.1005
TG(56:7)	0.15	0.0327	0.3281	0.3476	0.0662	1.63	0.1218	**1.97**	**0.0450**

TG(56:8)	0.15	0.0062	0.0282	0.9410	0.0180	2.24	0.0622	**1.82**	**0.0439**
TG(58:8)	0.14	0.0163	0.1217	0.5548	0.0275	1.60	0.1155	**1.98**	**0.0439**
TG(58:9)	0.15	0.0058	0.0180	0.8328	0.0151	1.86	0.0763	**1.75**	**0.0481**

Since 240 lipids reached $q<0.05$ at 8 weeks based on one-way ANOVA across the three groups, additional criteria were applied to limit the number of lipids for clarity. Only lipids with within-person changes in Lean or Fatty fish group significant at $q<0.05$ are included. No lipids were changed significantly within the Control group.

Author Conclusion:

The 8-week consumption of fatty fish decreased lipids which are potential mediators of lipid-induced insulin resistance and inflammation, and may be related to the protective effects of fatty fish on the progression of atherosclerotic vascular diseases or insulin resistance.

Reviewer Comments:

Small numbers of subjects in groups; groups appeared to have differences at baseline but baseline data was not statistically analyzed. Authors note that all subjects were using multiple medications which may have confounded the possible effects of fish consumption.

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions

1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions

1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes

1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	???
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	???
2.4.	Were the subjects/patients a representative sample of the relevant population?	???
3.	**Were study groups comparable?**	???
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	???
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes
4.1.	Were follow-up methods described and the same for all groups?	Yes
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	Yes
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	Yes
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	N/A

5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	Yes
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	Yes
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	Yes
7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes

8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	Yes
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes
8.6.	Was clinical significance as well as statistical significance reported?	Yes
8.7.	If negative findings, was a power calculation reported to address type 2 error?	N/A
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**	Yes
9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**	Yes
10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	Yes